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# Visual Experience Regulates Gene Expression in the Developing Striate Cortex

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Classification: Neurobiology

(critical period, neuronal growth associated protein GAP43, calcium/calmodulin dependent protein kinase II, glutamic acid decarboxylase, RNA blot hybridization)

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## ABSTRACT

We have examined the regulation of expression of the genes for the neuronal growth associated protein GAP43, the type II calcium/calmodulin dependent protein kinase, and glutamic acid decarboxylase in the kitten visual cortex during normal development and following a period of visual deprivation. We find that these genes, which display very different patterns of expression during normal development, are all up-regulated in the visual cortex of animals reared in the dark. Upon exposure to light, the expression of one of these genes drops to near-normal levels within 12 hours.

## INTRODUCTION

Under the guidance of normal visual experience, neurons in the striate cortex gradually acquire their mature levels of responsiveness and stimulus selectivity during a critical period of postnatal development which, in the cat, extends from 3 weeks to 3 months of age (1). Depriving kittens of light during this period retards the functional development of visual cortex, leaving cortical neurons poorly responsive to light and lacking normal selectivity. These effects of dark-rearing can be rapidly reversed upon subsequent exposure to light (2). Because these changes in cortical physiology can occur with only 6-12 hours of visual experience, it has been assumed that they reflect the "epigenetic" modification of synaptic connections (4). We set out to examine the possibility that patterns of gene expression in the striate cortex are affected by visual deprivation and light exposure. The results show that the effects of visual experience are not only manifested at the synaptic level, but are also revealed at the level of gene transcription. This introduces a new variable that must be considered in hypotheses about the modification of the cerebral cortex by sensory experience.

## MATERIALS AND METHODS

cDNA Probes. The cDNA probe for GAP43 is the clone pGA3A (5), which includes the entire human GAP43 coding sequence. The CaM kinase II cDNA, termed C23, is a 1.3 kb clone shown by sequence analysis to contain the carboxy terminal portion of the coding sequence of the $\alpha$ subunit of the rat type II calcium/calmodulin dependent protein kinase (C.-A. Ohmstede, personal communication). The GAD cDNA, termed GAD13, is a feline clone in pSP65 (6). The APP cDNA, termed HL124, has been described (7). The cDNA for MAP2, KN7, is a 2.4 kb insert representing the carboxy terminal portion of the coding sequence (8).

RNA Isolation and Blot Analysis. RNA was purified from 100-300 mg of tissue dissected from primary visual cortex, by the guanidinium thiocyanate procedure (9), with adaptations (10). The amount of RNA obtained from each region was determined by $OD_{260}$; $OD_{260}/OD_{280}$ ratios were also determined to confirm uniformity of this ratio among samples. Eight micrograms of RNA from each region was electrophoresed on agarose/formaldehyde gels, transferred to Biotrans membrane (ICN), and hybridized with radiolabelled probe as described (10). The blot was exposed to Kodak X-Omat AR film for 16 hr. For slot blot hybridization, RNA samples (500 ng) were vacuum dried, dissolved in 50 µl of 6.1 M formaldehyde in 10X SSC at 65° C for 15 min (1X SSC is 0.15 M NaCl, 0.015 M NaCitrate, pH 7.0), and brought to a volume of 200 µl with 15X SSC. Biotrans membrane was prewetted with 10X SSC and placed on a slot minifold apparatus (Schleicher and Schuell). Samples were loaded and vacuum applied. Filters were baked under vacuum at 80° C for 1 hr., and the RNA cross-linked to the membrane by exposure to UV light for 2 min. Hybridizations were performed as previously described for Northern blots (10). Radioactive signals from the slot blots were estimated with the LKB Ultroscan XL soft laser scanning densitometer. Areas under optical density peaks over a path encompassing the length of the entire slot were measured. Several exposures of each blot were made; those producing signals which fell in the range through which signal intensity was linearly related to the amount of RNA

loaded were selected for densitometric analysis. This linear range was previously determined with a standard curve constructed by blotting known amounts of ribosomal RNA and hybridizing the blot with a 28S rDNA probe.

Dark Rearing. Kittens and mother were transferred within a week of birth to a standard breeding cage in a dark room. Care was provided daily according to procedures approved by the Brown University Institutional Animal Care and Use Committee. Kittens used to study the effects of dark rearing were anesthetized in the dark prior to sacrifice and dissection. Animals to receive visual experience were removed from the dark and allowed to move freely on the floor of a lighted breeding colony for 6 hours from 12 noon to 6PM. The lights were turned off until 8AM the next morning, following which an additional 6 hours of visual experience was given prior to sacrifice.

## RESULTS

We chose to study the expression of the genes for the neuronal growth associated protein GAP43, the α subunit of the type II calcium/calmodulin dependent protein kinase (CaM kinase II), and glutamic acid decarboxylase (GAD). All three proteins are associated intimately with synaptic function in the neocortex. GAP43 is a neuron-specific phosphoprotein which is found in growth cones and some mature axon terminals, and has been linked with the development and functional modulation of synaptic relationships (11). Calcium/calmodulin dependent protein kinase II is concentrated in the postsynaptic densities of excitatory synapses (12,13), and is thought to play a critical role in the response to calcium signals generated by physiological activity or by extrinsic neuromodulators. GAD is the synthetic enzyme for γ-aminobutyric acid (GABA), and is concentrated in the axon terminals of inhibitory interneurons in the striate cortex (14).

We first surveyed the developmental expression of each of these genes in the visual cortex of normally reared kittens by Northern and RNA slot blot analysis (Figs. 1 and 2). Each cDNA hybridized to a single RNA species on Northern blots (Fig. 1): GAP43 to a 1.6 kilobase (kb) RNA, GAD to a 3.7 kb RNA, and CaM kinase II to a 5.0 kb RNA. The sizes of these mRNAs are consistent with those previously reported for the feline GAD (6) and for the rat GAP43 (5) and CaM kinase II (15) transcripts. Visual examination of the Northern blots revealed that each gene displayed a characteristic pattern of expression in the visual cortex during development. The abundance of the GAP43 transcript declined steeply during the immediate postnatal period, whereas the CaM kinase II transcript was initially present at very low levels and gradually increased during the transition to adulthood. In contrast, the level of the GAD transcript varied among time points without displaying a developmental trend.

These developmental changes in gene expression were quantified using slot blot analysis (results summarized in Fig. 2). The expression of the GAP43 gene dropped 8-fold from postnatal day 10 (P10) to adulthood; 85% of this decline occurred between P10 and P31. The expression of the CaM kinase II gene increased as that of the GAP43 gene fell: it was only 15% of adult levels at

age P10 but it rose to 40% of adult levels by P31, and to over 80% of adult levels by P56. The slot blot analysis confirmed the variability of GAD expression that had been suggested on the Northern blots. Most of this variability occurred among samples taken during the critical period, between the ages of P10 and P84 (Fig. 2). On average, however, GAD RNA levels increased only slightly from P10 to adulthood.

We next examined the effect of dark rearing and of subsequent exposure to light , on the expression of the GAP43, CaM kinase II, and GAD genes. RNA was isolated from the striate cortex of normally reared kittens (P43-50), from kittens reared in the dark since shortly after birth (P40-47), and from dark reared kittens that were exposed to 12 hours of light (P45-47). Levels of mRNA for each gene were quantified using slot blot analysis (Fig. 3A). We compared the expression of these genes to that of the microtubule associated protein 2 (MAP2) (8) and the Alzheimer amyloid protein precursor (APP) (7) genes, which were used as controls. In order to reduce the variance, all individual values were normalized against those obtained for MAP2, which did not change significantly under the conditions tested (Fig. 3B).

Levels of GAP43 mRNA were 80% higher in the visual cortex of dark reared animals than in that of normally reared animals of the same age group. This suggests that the high level of GAP43 mRNA normally seen in the early postnatal period may not drop as dramatically by P40-50 in dark reared animals as it does in animals reared normally. However, when dark reared animals were exposed to 12 hours of light, GAP43 gene expression dropped significantly to 125% of control levels. Expression of CaM kinase II and GAD were also elevated relative to normal in dark reared animals (175% and 200% of control, respectively), but the activity of neither of these genes changed significantly after 12 hours exposure to light. The expression of the Alzheimer amyloid precursor (APP) gene, shown here as a control, did not change significantly with dark rearing or with subsequent exposure to light.

## DISCUSSION

Converging lines of evidence suggest that GAP43 may play a central role in synaptic plasticity. For example, GAP43 is found transiently in axon terminals undergoing synapse formation (11) and changes in its state of phosphorylation correlate with the occurrence of long-term potentiation in the hippocampus (16). Indeed, we found GAP43 RNA in area 17 to be greatly elevated over the adult value at postnatal day 10, which is the onset of the most rapid phase of synaptogenesis in visual cortex (17). The steep fall in GAP43 gene expression over the next two weeks probably correlates with cessation of axonal outgrowth. Nevertheless, GAP43 RNA levels are still over 1.5x the adult value during the critical period. GAP43 RNA may persist in a subset of neurons specialized for synaptic modification during this period, with the secondary decline in GAP43 occurring in these neurons coincident with the end of the critical period. In any case, the decline in GAP43 expression during development evidently is delayed by dark rearing; but with 12 hours of visual experience, GAP43 expression falls to near-normal levels.

CaM kinase II is a major constituent of the postsynaptic density, and it has been suggested that its autophosphorylation may provide a synaptic mechanism for the long-term storage of information (18). Accordingly, increased expression of the CaM kinase II gene parallels the increase in synaptic density which occurs in striate cortex during the first 6 weeks of postnatal development (17). Given this correlation, it is somewhat surprising that CaM kinase levels are elevated in striate cortex of dark reared kittens. Similarly, Hendry and Kennedy (19) found in primate visual cortex that CaM kinase II protein levels are increased in columns deprived of normal input after monocular deprivation. These observations are consistent with the proposal that activity-dependent regulation of gene expression may provide a homeostatic mechanism to maintain synaptic effectiveness during periods of relative cortical inactivity (20). However, the level of CaM kinase II RNA did not decline in response to light exposure with the same rapidity as that of GAP43 RNA. Extension of the time of exposure to light will be necessary to define more precisely the temporal response of CaM kinase II expression to the initiation of visual experience

following dark rearing.

Inhibitory circuitry that uses GABA as a transmitter is thought to help shape the receptive fields and physiological response properties of visual cortical neruons (21). The extent to which this circuitry is modified by visual experience during the critical period, however, remains controversial (14). We find that the level of GAD expression in striate cortex is greater than 70% of the adult value at P10 and, on the average, increases only slightly during the critical period. However, dark rearing leads to a dramatic increase in GAD RNA which is not reversed by 12 hours of light exposure. This result contrasts with the absence of changes in GAD enzyme activity (14) or GABA receptor binding (22) in monocularly deprived kittens and the decrease in immunoreactive GAD observed after monocular deprivation in monkeys (23). Therefore, the increased expression of GAD in dark reared kitten striate cortex may be related to factors other than the level of patterned visual input activity, *per se*.

In summary, we have studied three genes in striate cortex that show distinct patterns of expression during normal development, but which are all more active in animals reared in darkness from birth. Hence, the genetic effects of dark rearing are inconsistent with the hypothesis that absence of sensory experience simply arrests normal visual cortical development. 12 hours of visual experience following 6 weeks of dark rearing, in addition to restoring normal physiological response properties to visual cortical neurons (2,3), also alters the rate of transcription of the GAP43 gene. However, the same period of time was insufficient to reverse the effects of dark rearing on the CaM kinase II or GAD genes, suggesting that cortical organization in dark reared kittens given 12 hours of light is not equivalent to that of normal kittens despite similarities measured electrophysiologically. Together, these data raise the possibility that regulation of gene expression plays an important role in the experience-dependent plasticity of the kitten visual cortex during the critical period.

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## FIGURE LEGENDS

Figure 1. Northern blot analysis of gene expression in the kitten visual cortex during postnatal development. The hybridization shown consists of selected lanes from a single blot that was hybridized to all three cDNA probes, labeled to identical specific activity (2 x $10^9$ cpm/ug), simultaneously.

Figure 2. RNA slot blot analysis of gene expression in the kitten visual cortex during postnatal development. All RNA's used for this slot blot analysis, and for the analysis described in Fig 3, were first hybridized with the cDNA probes on Northern blots to verify the integrity of the RNAs and the specificity of the probes. Each point represents an individual value, expressed as a percentage of the adult level. Note that the scale on the y-axis is different for each gene.

Figure 3. RNA slot blot analysis of gene expression in the visual cortex of kittens reared normally (n=6), reared in the dark (n=5), and reared in the dark and subsequently exposed to 12 hours of light (n=6). All values for 3A were normalized by dividing by the appropriate MAP2 signal. To verify the implicit argument that MAP2 gene expression does not change under the variable rearing conditions used, its pattern of expression is shown in 3B. Levels of MAP2 RNA did not change significantly in the visual cortex of animals reared in the dark or subsequently exposed to light.

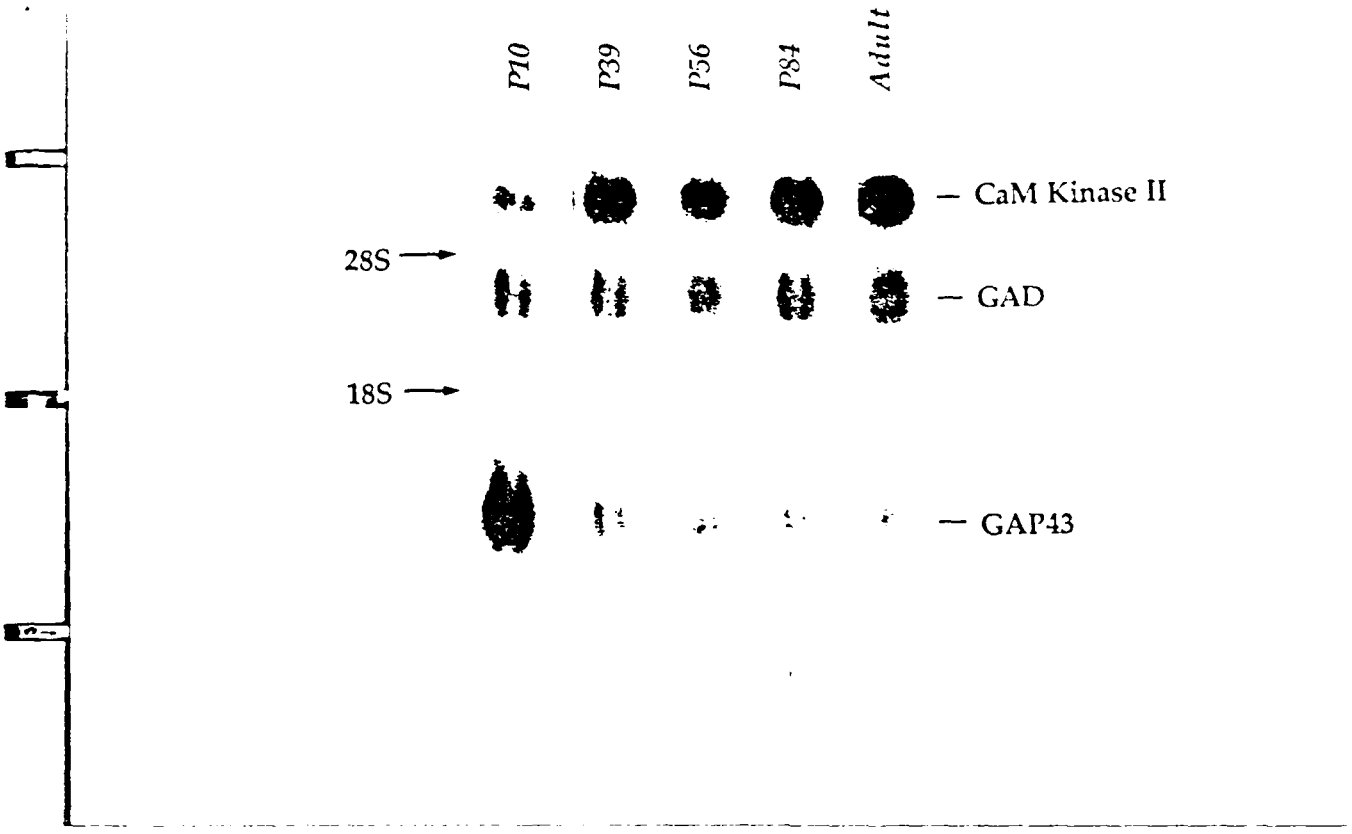


FIGURE 1.

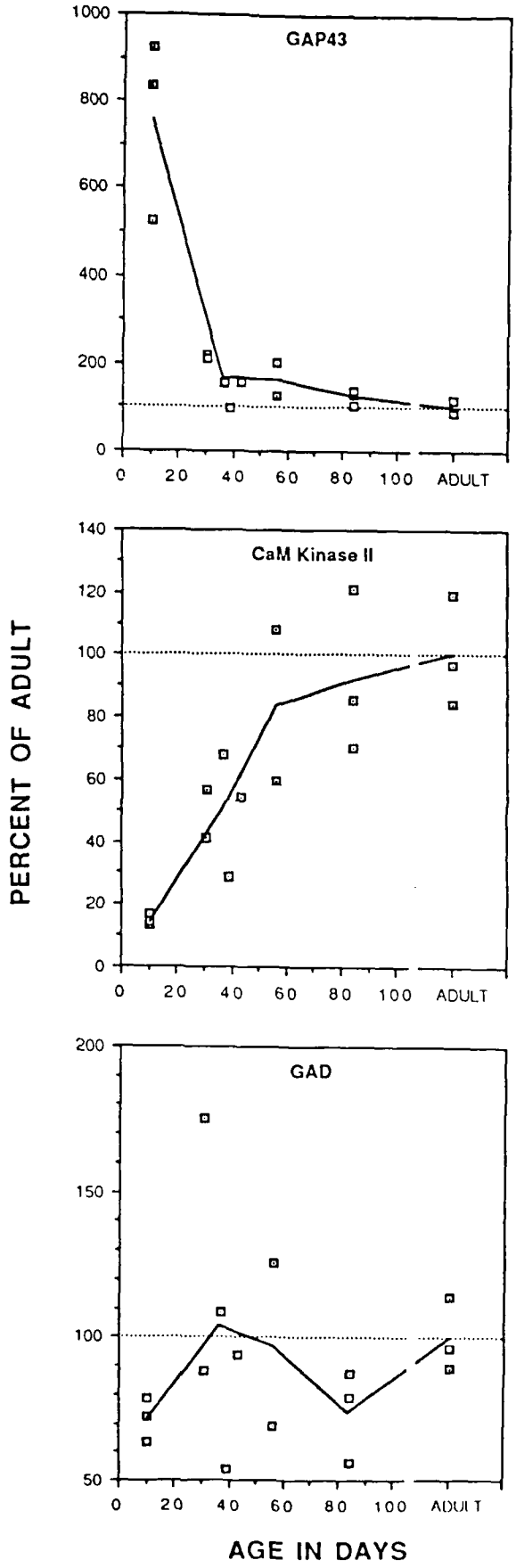


FIGURE 2

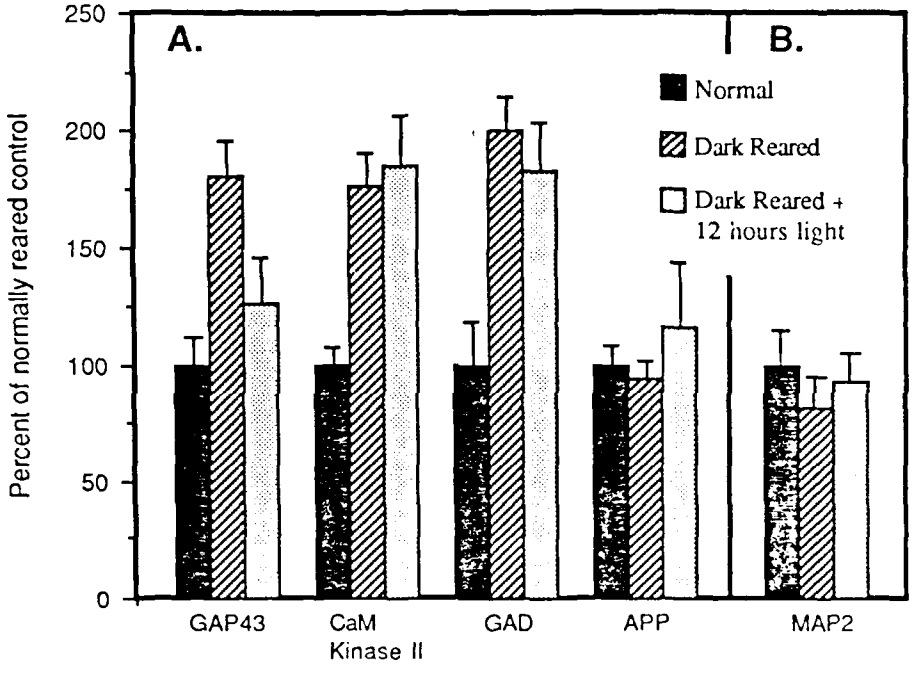
A.
B.
Normal
Dark Reared
Dark Reared + 12 hours light
Percent of normally reared control
250
200
150
100
50
0
GAP43
CaM Kinase II
GAD
APP
MAP2

FIGURE 3